The predominance of Ethiopian specific *M. tuberculosis* families and minimal contribution of *M. bovis* in tuberculous lymphadenitis patients in Ethiopia

By: Mulualem Tadesse (PhD scholar, Jimma University, Ethiopia)
Introduction

- TB is among the leading causes of morbidity and mortality in Ethiopia—ranks 10th in the world.
- Ethiopia has exceptionally high rates of extrapulmonary TB (EPTB).
- TB lymphadenitis (TBLN)—most common form of EPTB (accounting for 80% of EPTB).
- MTBc lineage/genotype vs TB disease presentation?

  - Studies reported an association between MTBc lineages and disease presentation [Caws et al., 2008; Kong et al., 2007, 2005].
  - However, Coscolla & Gagneux reviewed 100 articles and couldn’t find clear associations---??
Introduction…cont’d

- The epidemiological data on *M. tuberculosis* strains circulating in Ethiopia still remains largely unknown, particularly Southwest.

- Studies described the presence of Ethiopian-specific MTBc families and new phylogenic lineage (Lineage 7) in Northern Ethiopia
  
  [Biadglegne et al., Tessema et al., Firdessa et al]

- Whether or not the high rate of TB lymphadenitis in Ethiopia is linked to a specific MTBc lineage has not been systematically investigated.

- In this study, we have investigated:
  - the genetic diversity and epidemiology of MTBc strains in TBLN
  - the contribution of *M. bovis* in causing human TBLN
  - the role of newly described Ethiopian sub-lineages and /Lineage 7 in TBLN patients in Southwest Ethiopia
Methods and Materials

- Jimma, Southwest Ethiopia
- Patients with lymph node swelling and presumptive of TB (n=436)
- Lymph node (FNA) specimens
- Cytology (routine), Culture
- Xpert MTB/RIF—RIF resistance
Study work flow

436 FNA were cultured

- Culture negative (n=114)
- Culture contaminated (n=7)
- NTM (n=5)

310 culture positive MTBc

- Insufficient DNA quality (n=5)

305 genotyped by spoligotyping

304 included in the final analysis

uploaded on the [www.miru-vntrplus.org](http://www.miru-vntrplus.org)
Classification of strains into lineages and sub-lineages

- Spoligotyping & MIRU-VNTR patterns were double entered
- Uploaded on the www.miru-vntrplus.org
  - The best match with strains of the reference database (cut-off 0.17)
- Ethiopian specific families /Lineage 7 were new to the database
  - Phylogenetic tree (GMA tree) based identification
  - Lineage-specific SNP analysis
    (Biadoglegne et al.; Tessema et al., Firdessa et al.)
Results and Discussion

- TB lymphadenitis in Southwest Ethiopia is caused by different genotypes of MTB
  - Ethiopian specific sub-lineages within Lineage 4 (36%)
  - Lineage 3/Delhi-CAS (15%)
  - Lineage 4-Haarlem (14%)

- Lineage 4 is the most dominant
  - The ill T family and Haarlem
  - >90% of T-family—were being part of the new defined Ethiopian families
    - Ethiopia_2, 3 and H37Rv-like
  - MTB genotype distribution is similar with previously reported from pulmonary and TBLN in Ethiopia
Phylogenetic tree Ethiopian specific families/lineages

Ethio_H37Rv-like L4
Ethiopia_2 (L4)
Ethiopia_3 (L4)
Lineage 7/Ethiopia_1
Results and Discussion

Ethiopian lineage (Lineage 7)
- 6 isolates (2%) with unusual spoligotype patterns (missing spacer 4-24)
  - New to the database & have typical spoligotype
  - Contributing a new phylogenic lineage---- called Lineage 7
- This is one of the first study to document L7 in Southwest Ethiopia

- L7 were first discovered in Northern Ethiopia & limited to the Horn of Africa
- Studies in Northern part of Ethiopia reported higher contribution L7
  - Biadiglign et al and Tesema et al -- reported (8-10%) vs 2% in ours
- The restricted geographic distribution of L7 could be explained by
  - L7 has a lower rate of progression to disease relative to others
  - Have a host preference for ethnically Ethiopian people
Results and Discussion

- **Lineage 3 (Delhi/CAS)** is the other dominant lineage in this study
  - Supposed primarily found in Central Asia
  - Delhi/CAS is found across the different regions of Ethiopia, indicating the successful spread of these strains
    - (Biadglegne et al.; Tessema et al. Ali. et al., 2016)
    - ✓ through human movement, Central Asia to Ethiopia

- **Lineage 3 (Delhi/CAS)** is also associated with Rifampicin resistance
  - 8 of 10 RIF-resistant strains, belonged to Delhi/CAS (L3)
  - Six of these formed two clusters and shared their RIF-resistance conferring mutations at *rpoB* gene (codon 447–452),
    - ✓ thus possibly suggesting recent transmission of RIF-resistant strains
Role of *M. bovis* in TBLN in Ethiopia

- *M. bovis* was identified in only 0.7% (2/304)
- Typical bovine spoligotype patterns (SIT 665) lacking spacers 3–5, 9, 16, and 39–43
- Similar to overall data in Ethiopia: 0.4% of *M. bovis* in TB patients (*Firdessa et al. 2013*)
- These findings indicate that the overall contribution of *M. bovis* to human TBLN infection is minor
- In general, declining rates of *M. bovis* from human TB patients have been reported in Ethiopia,
  - may be due to increased practice of pasteurizing milk and milk products

### Prevalence of Bovine TB is high in Ethiopia

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lineage1</td>
<td>3</td>
</tr>
<tr>
<td>Lineage2</td>
<td>1</td>
</tr>
<tr>
<td>Lineage3</td>
<td>44</td>
</tr>
<tr>
<td>Lineage4</td>
<td>248</td>
</tr>
<tr>
<td>Lineage7</td>
<td>6</td>
</tr>
</tbody>
</table>
## Results and Discussion

### Clustering rate

<table>
<thead>
<tr>
<th>Methods</th>
<th>No. profiles</th>
<th>No. of isolates with unique profiles</th>
<th>No. of clusters</th>
<th>No. of isolates in cluster</th>
<th>Clustering rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spoligotyping</td>
<td>43</td>
<td>28</td>
<td>15</td>
<td>138</td>
<td>74.1</td>
</tr>
<tr>
<td>MIRU-VNTR</td>
<td>108</td>
<td>84</td>
<td>24</td>
<td>82</td>
<td>34.9</td>
</tr>
<tr>
<td>Spoligotyping + MIRU-VNTR</td>
<td>117</td>
<td>95</td>
<td>22</td>
<td>71</td>
<td>29.5</td>
</tr>
</tbody>
</table>

- The clustering remains very high for **Ethiopia_3 isolates (T3_ETH)** which is in agreement with another study in Northern Ethiopia:
  - 17 Ethiopia_3 isolates were clustered (identical spoligotype and MIRU-VNTR) with 10 of Ethiopia_3 isolates from Northern Ethiopia
- Ethiopia_3 strains remain the predominant source of the most recent infection for TBLN cases--though no epidemiological link
Conclusions

- TB lymphadenitis disease presentation does not seem to be linked to a specific MTBc lineage type/genotype
- The Ethiopian specific sub-lineages within Lineage 4 are the most dominant
- We documented presence of lineage 7 in Southwest Ethiopia
- Contribution of *M. bovis* in TBLN infection is minimal
- The cluster rate was highest for Ethiopia_3 isolates showing clonal similarity with isolates from North Ethiopia
- A more detailed genetic analysis of locally contained Ethiopian specific sub-lineages by whole genome sequencing is warranted
Acknowledgements

Study participants
THANK YOU